Author's response to reviews

Title: Associations between gestational anthropometry, maternal HIV, and fetal and early infancy growth in a prospective rural/semi-rural Tanzanian cohort, 2012-13

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Author's response to reviews: see over
Dear BMC Pregnancy & Childbirth Editors:

We thank you for the opportunity to submit a revised version of our manuscript entitled, “Gestational mid-upper arm circumference and birth or early infancy anthropometrics in HIV-exposed and unexposed infants: a prospective cohort”.

We have summarized point-by-point responses to each reviewer comment indicated below, as well as noting corresponding changes in the revised manuscript.

We are grateful to the reviewers for their thoughtful comments, and by addressing each of their comments, we feel that the revised version is an improved communication of this study.

Sincerely,

Joann M. McDermid

Reviewer’s report (#1)
Reviewer: Andreas Chiabi

Major Compulsory Revisions
1. The title does ties poorly with the contents of the article or the objectives of the study. If focus is on MUAC, what was the use of the other anthropometrics in the mother?

The title has been changed to more broadly reflect the objective to identify maternal risk factors associated with fetal and early infancy growth with potential to measure in a non-urban resource-restricted setting. The original title focus on MUAC was selected to reflect the hypothesis that MUAC would be the key gestational measurement given its previously demonstrated relationship with LBW and point-of-care characteristics, even though a priori we were uncertain whether it would also be a risk factor for birth length early infancy growth in this setting. In the study design, maternal height, weight, BMI were also included as they would provide some indication of the broader population characteristics (i.e. maternal height would likely indicate a history of health impacts, rather than current gestational circumstances) and in relation to other populations. Additionally, weight, height and BMI are part of routine ANC measurements, even if weight is often the only measurement routinely recorded in this setting. TSF was included even though it is unlikely that skinfold calipers and training would be available in this setting since this is a marker of maternal adiposity that may independently predict growth. Given that the broader measurements selected and the findings reported (i.e. that both MUAC and HIV status are important), we have changed the word MUAC to “gestational anthropometry” and included HIV and additional descriptors to indicate the cohort setting (i.e. Tanzanian rural/semi-rural) and time.

2. The Abstract should take into consideration the modifications in the final corrected text.

The abstract corresponds to the revisions made in the final text.

3. Line 83: It should be well precised here, if the use of MUAC especially, and triceps skin fold to assess the nutritional status in pregnancy is a WHO recommendation or any scholarly society; if not, have other studies confirmed validity of MUAC in assessing the nutritional in adults or pregnant women??
There are no consensus guidelines established to identify nutritional risk in pregnant women in rural resource-poor settings in relation to birth anthropometrics, and the extension to early infancy has not been made. In a recent report, Tang, AM. et al. 2013. Use of Cutoffs for Mid-Upper Arm Circumference (MUAC) as an Indicator or Predictor of Nutritional and Health-Related Outcomes in Adolescents and Adults: A Systematic Review. Washington, DC: FHI 360/FANTA, summarized that MUAC in the range of 21 to 24 cm was associated with poorer birth anthropometrics. This report indicated that, “Taken together, the results suggest that low MUAC can be used to identify individuals at increased risk of adverse outcomes across adolescents, adults, the elderly, and pregnant women, but there is insufficient evidence on which MUAC cutoff optimizes sensitivity and specificity for any particular diagnostic criteria or population.” In another specific setting, a humanitarian context, MUAC cut-offs of <23 cm have been suggested as informative on the basis of the relatively strong association with LBW, narrow range of cut-off values, simplicity of measurement and since it does not require gestational age ascertainment: Ververs M, et al. PLoS Curr. 2013 June 7; 5: ecurrents.dis.54a8b618c1bc031ea140e3f2934599c8.

This paragraph has been modified in Lines 82-9 to explicitly state that MUAC is widely used to identify pediatric malnutrition and to a lesser extent to identify malnutrition in pregnant and lactating women, but that there is no universal WHO recommendations. We indicate that one NGO is recommending maternal MUAC to screen for LBW, and link their rationale for recommending it to the rural Tanzanian setting this cohort was based in.

4. Line 85: Hb is not anthropometry
Hb has been deleted.

Methods
5. Line 100: What was the rationale for the GA limit of eligibility < 34 weeks? What happened to those pregnancies that did not go to term? Enrolling them would mean enrolling extremely premature neonates who will not have had their catch up growth pattern and normal anthropometrics as for term neonates at birth and by 6 months the duration of the study. The eligibly cut-off was actually <36 weeks gestation and this has been corrected from the <34 incorrectly indicated in the previous version. This cohort included about 30% of women with preterm deliveries based on their estimated gestational ages. Gestational age was likely estimated with inaccuracy, although the true number is difficult to estimate in studies without access to ultrasound technology. Gestational age was considered in statistical analyses.

6. Concerning ethics, which interventions and health care services were offered to women found to be malnourished and enrolled in the study.
Participants (mothers or their infants) in this observational study that were identified as malnourished or requiring additional diagnosis and clinical follow-up for any condition (e.g. anemia) were referred via the study physician liaison to the main clinical services of the Kisesa Health Centre, where the research took place. Additionally, at the end of the study, a dissemination day was held where all participants were provided transportation funds to return to the clinic for an afternoon where study findings were communicated, and general nutritional guidelines for mothers and best practices for infant feeding and health were offered.

7. Lines 103-104: The sentence 'For every HIV-positive woman enrolled, the recruitment goal included two HIV-negative women”, is ambiguous and unclear. This should be clarified. This section has been modified to clarify in Lines 117-20.

8. Is this study a case-control study or not? The study design should be mentioned and stated in
the Methodology
This study was a prospective cohort, and the manuscript has been modified to include the design in the title, the abstract (Line 28) in the methods (Line 98).

9. How was the sample size determined??
Please refer to explanation to point 3 of reviewer 3.

10. Line 104: At what stage or GA were women considered to sero-negative? At enrollment (<34 weeks)? if so there is a considerable bias, as some pregnant women can be sero-negative at early or mid pregnancy and get infected at late pregnancy.

Women were tested for HIV upon enrollment in the study, as part of standard antenatal care at the host clinic. Many women attend only a single antenatal visit in this region, less than 50% typically deliver at a clinic and post-partum care of mothers to include HIV retesting is not routine, therefore the likelihood of subsequent seroconversions throughout pregnancy or during lactation in this study is unknown. Without knowing these rates, we are unable to comment on the extent of potential bias of subsequent seroconversions that occur between the median gestational age at enrollment and delivery spanning about 10 weeks for (assumed) HIV-negative women. While HIV seroconversions during this period would clearly have health implications for PMTCT and infant HIV-related care, it is unclear what implications seroconversion during the latter 10 weeks of gestation would have on birth and early infancy growth measurements. The term “at enrollment” was added to the criteria of known HIV status in Line 115.

Data collection

11. Line 128: Maternal anthropometric measurements were taken measured at what gestational ages?
This has been clarified in Lines 161-3.

12. Line 141: What was the MUAC cut-off for malnutrition in the pregnant women, and infants (considering the GAs at birth) in this study?
In the absence of a universally accepted cut-off for maternal MUAC that is predictive of infant outcomes during pregnancy, we examined relationships between maternal MUAC and outcomes based on the distribution of our study data (tertiles). Likewise, MUAC references do not exist for infants ≤ 6 months and we choose to analyze infant MUAC as a continuous variable, adjusting for gestational age.

13. Line 145: Define the other feeding operational terms: partial and predominant breast feeding
This has been defined in Lines 183-5.

14. Line 213: Was any correlation established between MUAC degree of immunodepression and the infant’s anthropometrics/outcome??
Examining these variables did not indicate any associations in this data set, but it may be in part due to the fact that almost half of HIV-positive women had normal CD4 levels (>500), 75% with CD4 above 350, and there were only 4 women with advanced immunosuppression (CD4<200). In a study powered to detect differences at all levels of immunosuppression, a different picture is biologically plausible, and probable.

Statistical analysis

15. Line 164: replace the URL with the appropriate reference.
The macro package is often cited as a URL in manuscripts to enable direct access to the macro
A few published examples listed below include the same citation:


I am not aware of the use of 'wasting' and 'stunting' in infants less than 6 months old. This should be clarified.

We agree that 'stunting' and 'wasting' are more commonly used in infants >6 months old since the manifestations of these conditions may be more likely to present at later ages. However, these terms have been used to describe infants from birth and at ages <6 months old. A few published examples are listed:


The term 'wasting' has been replaced with 'underweight' throughout the revised manuscript, as no infant was identified with wasting (<-2 SD WHZ), while infants were classified as underweight (<-2 SD WAZ) using the WHO/UNICEF definitions.

16. Lines 251-254: This should be discussed with other findings from other studies
This has been modified in Lines 304-9 to include two review articles that summarized African studies of post-natal growth in HIV-positive and HIV-exposed infants compared to HIV-unexposed in urban settings.

17. Line 271: Reference??
This referred to the current study and the sentence has been modified in Lines 325-8 to clarify this.

Results
Precise how many seropositive and seronegative pregnant women were enrolled in the study
The number of women enrolled according to HIV status has been indicated in the first line of the Methods (Line 98), can be inferred in the Abstract (Line 31) and the modified Figure 1 that shows the number enrolled and study exits of mothers and their infants according to maternal HIV status.

Minor Essential Revisions
Under Background
1. Line 70: ……the pregnant women
Corrected in Lines 72-4.

Results
2. Lines 189-191: report your results without any discussion nor justification. The discussion will
be done later.
Sentence modified in Line 235-7.


Discussion
4. Line 246: 6 months is not 'long term'
Sentence modified in Lines 296-8.


Reviewer’s report (#2)
Reviewer: Kathleen Powis

Major Recommendations:
1. In the BACKGROUND section of the ABSTRACT, the authors indicate that rural populations represent an understudied group. While they likely represent a group with poor access to a sound health care infrastructure, there are numerous citable studies of pregnancy outcomes and maternal nutritional status in rural settings within developing countries.

This needs to be either re-characterized to reflect the often limited healthcare infrastructure found in rural settings, multiple citations that substantiate the assertion need to be provided or the suggested research disparity needs to be removed all together.

Of note, often the INTRODUCTION of a manuscript will echo key comments made in the BACKGROUND section of a manuscript. There is no reference to rural areas being “understudied” in the INTRODUCTION. Please correct this reference in the DISCUSSION section of the manuscript (lines 246 and 305).

We have changed the abstract to read: “Healthcare access and resources differ considerably between urban and rural settings making cross-setting generalizations difficult. In resource-restricted rural/semi-rural environments, identification of feasible screening tools is a priority.”

The reported finding applies to any setting, thus the reference to understudied rural populations has been removed in Lines 296-8 (corresponding to earlier version line 246).

Likewise, understudied has been removed from the limitations section (corresponding to earlier version line 305) in Lines 368-70 as the point that despite the large proportion of people living in non-urban settings in SSA, conducting medical research is more challenging in this setting can be made without this reference.

2. In the METHODS section of the ABSTRACT it is stated that “Mid-gestation” anthropometry was obtained. However there is no definition of “mid-gestation” in the manuscript. Table 1 indicates that women were enrolled as early as 16.6 weeks gestation and as late as 34.9 weeks gestational age. It may be more appropriate to define the timing of maternal anthropometric measurements as either within the 2nd or 3rd trimester or specifically define the minimum and maximum gestational age at which maternal anthropometry was obtained.

Modified to indicate women were recruited in their second or third trimester (Lines 31-3), and the min/max gestations added to the Results section, Line 226.
3. In the RESULTS AND DISCUSSION section of the ABSTRACT, the opening line indicates that "gestational mid-upper arm circumference and HIV status (HIV-positive=39%) were prognostic determinants of infant weight-for-age and length-for-age z-scores from birth to 6 months, even after adjustment for infant feeding practices". First, this is a prospective observational study. Therefore, causation cannot be established, but an association was found. The term "prognostic determinant" has been removed from the abstract and manuscript text.

Secondly, "HIV-status" should be modified to indicate “maternal HIV-status”. While the prevalence that follows implies a maternal HIV status and the methods section will provide the reader with the fact that HIV-infected infants were excluded, the abstract should be clear on its face without the reader needing to delve farther into the manuscript. HIV-infected infants were not excluded, however, no infant tested positive at 3-months of age so the cohort did not include HIV-infected infants. Abstract and main text wording has been modified to indicate maternal HIV status where appropriate.

Lastly, reference is made here and throughout the paper to “gestational mid-upper arm circumference”. Other literature on this topic refers to “maternal mid-upper arm circumference”. This may be a term better understood by readers. Maternal MUAC is used more often, however, the term maternal can apply to the gestational and/or post-partum periods. Since the measurement of anthropometry in mothers only occurred during pregnancy while mothers were followed throughout the 6-month post-partum period in this study, we chose to use the more specific gestational anthropometry terminology. The term gestational anthropometry is retained in the manuscript when referencing the measurements/results of this study, and the term “maternal” included in the key words so a search on “maternal” and “anthropometry” should also capture this study reference.

4. In the RESULTS and DISCUSSION section of the ABSTRACT, it is indicated that gestational mid-upper arm circumference was significantly associated with "overall" infant weight-for-age and length-for-age z-scores, with respective effect estimates of +0.11 for both outcomes. This finding needs to clarify that in linear mixed effect models for WAZ and LAZ from birth through 6 months of life, each 1 cm increase in maternal MUAC was associated with +0.11 increased infant WAZ and LAZ.

Modified as suggested, Lines 44-6.

5. In the CONCLUSIONS section of the ABSTRACT, it is stated that “In rural settings, HIV-exposure is associated with poorer anthropometry and growth faltering throughout the first 6 months, despite improving antiretroviral access and better infant feeding practices. It would be more factual to state that "In our rural and peri-rural setting", as not all rural settings have improved antiretroviral access and better infant feeding practices.

The first part of the sentence has been modified as suggested, Lines 51-2. In the second part of the sentence, the phrase "better infant feeding practices" has been removed. While HIV-positive women exclusively breastfed their infants for longer, the majority of women were not feeding their infants according to recommendations. While statistically accurate and possibly clinically relevant, without the ability to provide a fuller explanation of “better” in the abstract may imply more favorable infant feeding practices occurred than those that actually were reported.

Secondly, reference to growth faltering is made in this statement but the term is not defined in the METHODS, RESULTS or CONCLUSIONS sections of the manuscript. Unless this is expanded upon, this subjective term should be eliminated from the abstract.
The term “growth faltering” has been removed.

6. In the BACKGROUND section, the final paragraph talks about use of maternal MUAC in humanitarian crises settings and states that it is rarely used in antenatal settings. You may want to refer to: http://www.cmamforum.org/Pool/Resources/MUACcutoffs-for-adols-adults-Systematic-Review FANTA. Of note, it may be particularly important to highlight that although maternal MUAC has been reported in the past relative to birth outcomes, no reference ranges exist. The background section has been revised to include these points, Lines 80-95.

7. In the METHODS section at line 100 it indicates that study eligibility included gestational age of < 34 weeks. However, in table 1, the standard deviation of maternal gestational age at enrollment indicates that some HIV-infected women were enrolled at least as late as 34.9 weeks. Please clarify the enrollment criteria.

Thank you for noting this error, the correct gestational age cut off was <36 weeks. We have corrected this portion of the METHODS section by replacing the number “34” with “36”.

8. The final sentence in the STUDY POPULATION portion of the METHODS section indicates that all infants remained HIV-negative. It is somewhat surprising that all infants were able to leave their rural or semi-rural setting, present to a regional hospital and have their PCR results returned to them in a timely manner. This would not be in keeping with the normal fall-out in the cascade of care of HIV-exposed uninfected infants and is somewhat surprising, as not all mothers were on triple antiretrovirals. Can you please confirm that definitive results were available on 44 infants at 3 months of life and non-breastfed beyond 3 months of life?

This information was obtained from the medical charts of all infants who were in the cohort at 3-months of age (32 out of the original 44 with birth data). 6 exited the cohort prior to month-3 as indicated in Figure 1, and HIV-test results were unavailable for them. Kisesa Health Centre (where the study took place) is where mothers would return with their infants to have the blood drawn for infant testing. The samples, but not the infants, were sent for testing using dried blood spot HIV DNA PCR at the regional hospital, Bugando Medical Centre, as is standard procedure.

Women in this study were provided transportation costs as part of their cohort enrollment to attend study visits, including one at 3-months, and this may have facilitated the testing coverage of study participants. In addition, Kisesa Health Centre has been the site of considerable HIV demography and serosurveillance studies, and this may have also influenced study coverage and testing linkages that have already been established between Kisesa and Bugando. Additionally, Tanzania has achieved considerable success in vaccine coverage, even in rural areas, and the 3-month testing corresponded with the infant vaccine schedule. Together, these factors may have led to successful return to clinic for infant HIV testing at 3-months.

Infants were not re-tested for the study-specific visit at 6-months, and it is possible that there may have been seroconversions between 3 and 6-months. The text in the manuscript indicated testing was done at 3-months, but an additional qualifier has been added in Lines 151-3 to indicate all infants were HIV-negative at month-3.

9. In the DATA COLLECTION portion of the METHODS section or the RESULTS section, please provide the percentage of gestational ages that required estimation of fundal height due to the fact that the mother could not provide an LMP. The percentage (6%) has been added in Lines 156-60.
10. In the DATA COLLECTION portion of the METHODS section, the scoring system for infant feeding practices is presented. However, further definition is needed around “1=partial breastfeeding” and “2=predominant breastfeeding”.

The study-specific definitions for partial and predominant have been included in Lines 183-5.

Secondly, please clarify, since this score was developed to compare infant feeding practices at each visit, if an infant could receive a score of 2 at the one month visit and a score of 3 at the two month visit, as an example or was a score assigned once to the first six months of life. This is correct, and to clarify in the methods we have included scoring examples with an infant transitioning at different months with different infant feeding practices Lines 187-92.

Just to clarify, an infant who exclusively breastfed through six month of life would have had a score of 12, or 3 at each of the 4 study visits after birth?

This is correct, and to clarify in the methods we have included this as a scoring example Lines 187-9.

11. In the STATISTICAL ANALYSIS portion of the METHODS section, it states that infant anthropometrics at birth and gestational clinical characteristics were analyzed using simple linear regression, adjusted for maternal HIV status, age, parity, education, gestational age at the time of anthropometric measurement and infant gender. However, in reviewing Table 2 and 3, it appears as if univariate linear regression was performed as a first step followed by multivariate linear regression. This should be clarified in the METHODS section.

This has been revised in the Methods in Lines 199-202.

12. In the STATISTICAL ANALYSIS portion of the METHODS section, MUAC tertiles and maternal gestational MUAC as low or high are discussed. Please clarify that for MUAC tertiles, cut-offs were based on MUAC findings of the cohort and that for the dichotomous variable of low or high gestational MUAC, the midpoint of MUACs in the cohort was selected. It would also be helpful to clarify the exact tertiles and the dichotomous breakpoint in results section. Of note, the results section references low vs high MUAC tertile comparisons. So if this is not a dichotomous cut, it really needs to be better explained in the METHODS section.

The approach for categorization of cutoffs in Methods has been described and reporting of results with corresponding tertile cutoffs indicated has been included throughout the revised manuscript.

13. In the STATISTICAL ANALYSIS portion of the METHODS section, reference was made to adjustment of the mixed effects models for potential confounding variables. However, the approach, whether a priori inclusion of potential confounders or reliance of a p-value in excess of 0.05 to reintroduce a covariate into the mixed effects model is not described. Please clarify the approach.

Covariates were chosen a priori. This has been clarified in Lines 199-202.

14. In the MATERNAL CHARACTERISTICS portion of the RESULTS section, it is indicated that HIV-positive women enrolled significantly earlier in pregnancy. However, Table 1 indicates that they enrolled significantly later. Is the error in the text or in table 1? Also, in the DISCUSSION section on page 13, at line 250, it indicates that HIV-exposed pregnancies received earlier antenatal care. However, this is not portrayed as such in Table 1.

The error whereby gestational ages listed in Table 1 were mistakenly switched between groups has been verified and corrected.

15. In the MATERNAL CHARACTERISTICS portion of the RESULTS section, the text reads
that HIV-positive women were not significantly different from HIV-negative women in their health according to anthropometric indicators. How does anthropometric measures dictate health? This was not meant to imply that anthropometrics dictate health, but rather that anthropometrics are dictated by health as a function of an individual achieving sufficient dietary intake and metabolic utilization in relation to requirements.

Were they equally likely to be hypertensive, anemic or have TB based on comparable anthropometric measures? Data was presented in this manuscript on anemia (Lines 231-5) demonstrating no differences according to HIV status in terms of mean Hb or severity. Coinfections and comorbidities were not actively investigated, and therefore cannot be ruled out, however, even if more common in HIV-infected women, the consequences were not yet apparent in maternal Hb or anthropometrics.

It does not appear that health data was collected in order for this statement to be made. Any adjustments made to this wording should also be applied to the final sentence of the manuscript, which makes the same assertion. The wording of the statement was qualified by the data that was collected as it was in the same sentence as the results for Hb reported, and the qualifying words “...in their health according to anthropometric indicators” was included in the original submission Lines 186-188. This has not been modified. The final sentence has been modified in Lines 392-6 to include the qualification of Hb concentration and anthropometrics when considering health status.

16. In the MATERNAL CHARACTERISTICS portion of the RESULTS section there is discussion of “ART”. It is not clear whether “ART” references both AZT monotherapy and triple therapy. Given findings that triple antiretrovirals taken in pregnancy result in decreased birth weight and are associated with lower longitudinal length-for-age z-scores, it is very important not to lump AZT-monotherapy and triple ARVs into a common category. References to ART as a general term for any therapy or to specific therapies where relevant have been clarified throughout the manuscript.

17. In the PREGNANCY OUTCOMES and BIRTH ANTHROPOMETRICS portion of the RESULTS section, it is stated that suboptimal pregnancy outcomes were common with 5% of pregnancies ending in stillbirth or miscarriage. It would be more appropriate to simply report the proportions without subjective characterization of the proportions. If these proportions are “common”, either relative to rates in Tanzania or globally, it would be more appropriate to make this connection with appropriate citations in the DISCUSSION section of the manuscript. Modified as suggested, Lines 248-9.

18. There is reference to prematurity in the manuscript. The more appropriate term is “preterm delivery”. An infant can be born on or after 37 weeks gestational age, yet have a physical exam that is consistent with prematurity. Please correct this terminology.

Modified as suggested throughout the manuscript.

19. In the PREGNANCY OUTCOMES and BIRTH ANTHROPOMETRICS portion of the RESULTS section, it is reported that HIV-positive mothers delivered small babies and it then goes on to report “birth weight” as -235 grams. This is not a birth weight, but rather the expected difference in birth weight compared to an infant born to an HIV-uninfected mother. Furthermore, this is only in univariate analysis. In multivariate analysis the difference in birth weight indicates that infants born to HIV-infected mothers would be expected to weigh 281 grams lighter, after adjusting for other covariates. This is the result that should be reported. It is not clear that the univariate model/results are needed.
As suggested, the wording has been modified in Lines 256-62, and the multivariate result only reported in the text. Univariate results are presented in Table 2 since there is limited recent data from similar contexts, and presenting multivariate analyses only may obscure the comparability if studies did not measure or adjust for covariates in the same way as this study.

20. In the PREGNANCY OUTCOMES and BIRTH ANTHROPOMETRICS portion of the RESULTS section, there is reference made to a dose-response relationship between MUAC tertile and birth length. This is not dose-response, but rather a positive association with increased birth length noted at each higher maternal MUAC tertile. This has been modified as suggested in Lines 255-8.

21. In the INFANT GROWTH portion of the RESULTS sections, several references are made to “strong associations”. It would be more appropriate to characterize these as “was found to be statistically significantly associated with...”. The term “strong” has been removed throughout the revised manuscript.

22. In the DISCUSSION section starting at line 251, it is indicated that evidence from this study demonstrate that HIV-exposed pregnancies and infants continue to have poorer prognosis than HIV-unexposed infants in terms of birth and growth outcomes in this rural setting, despite improving antenatal HIV-testing, maternal ART and PMTCT implementation. There is no indication in this paper that HIV-infected women either conceived on triple ARV regimens or were started in the 14th week of gestation to indicate that this cohort had optimal PMTCT treatment. It is reasonable to point out the worse outcomes experienced by HIV-exposed infants, but not to target it to better PMTCT practices in this cohort. Reference to PMTCT has been removed. As a minor point, reference to HIV-exposed uninfected infant is probably a better overall description than HIV-exposed pregnancies and infants. Modified as suggested in Lines 302-3, 306 and 394.

23. In the DISCUSSION section starting at line 288 it indicates that while maternal HIV care continues to improve and important reductions in maternal HIV transmission have been achieved in Africa, the findings of this study suggest that HIV-exposed pregnancies and infants living in this rural and semi-rural setting still have a poorer growth outcomes compared to HIV-unexposed infants. This comment fails to take into account the contribution of the ARVs to which a portion of the infants were exposed in utero. Studies have found that triple ARVs compared with mono-therapy are associated with significantly lower infant birth weight and longitudinal LAZ. Therefore, in this section, it would be more appropriate to acknowledge that while in this cohort, models were structured to compare maternal HIV-status, they were not able to isolate the impact of in utero exposure to ARVs. A sentence in Lines 347-9 has been added in the Discussion to address this important limitation.

24. On line 297 of the DISCUSSION section, it indicates that the study data extends further by demonstrating that maternal HIV status was a risk factor for short birth length. This is not a new finding and has been cited in the literature from sub Saharan Africa. Discussion text revised to reflect this in Lines 354-6.

25. In the DISCUSSION section on line 303, it indicates that HIV-positive mothers exclusively breastfed for a significantly longer duration than HIV-negative mothers. While there was a statistically significant finding, the fact that HIV-uninfected mothers only exclusively breastfed for
a mean of 3.4 weeks and HIV-infected mothers only exclusively breastfed for a mean of 6.6 weeks, it is well below WHO recommendations and breastfeeding practices in sub-Saharan Africa and both would be expected to impact growth. Given this very short window of exclusive breastfeeding, it would be important to provide the reader with mean weeks of exclusive breastfeeding, as well as mean weeks to breastfeeding cessation in the RESULTS section of the paper. Additionally, it should be pointed out in the limitations paragraph, that findings may not be generalizable to other settings, particularly rural settings in sub-Saharan Africa, where EBF is generally sustained for longer periods that approach six months of life, particularly in infants of HIV-uninfected women.

Without a universally used definition of breastfeeding practices, including for EBF, we would argue that the true duration of EBF is unknown in sub-Saharan Africa. Many studies indicate they are measuring EBF, but upon closer examination, it is apparent they are not using the WHO EBF definition that we used, and in fact, other non-medicinal liquids have been included or not even inquired about. Given this mixture of breastfeeding practices that are termed “EBF” in the literature, it is possible the true duration of “EBF” in Sub-Saharan Africa is considerably shorter than currently reported. We have modified the limitations section to note that the EBF is much shorter than recommended in this study, and note the limitation in generalizability of findings between studies not using the same EBF definition in Lines 361-7.

26. In the DISCUSSION section the reader is provided, for the first time, with the fact that complete birth anthropometric data was unattainable within the 72 hour period after delivery for 24% of the infants, a large number. This needs to be reported in the results and also needs to be reported by maternal HIV status. It cannot be discerned from Table 1 whether both exposure groups were affected by missing data.

Birth anthropometric data availability first appeared in the Methods section in reference to Figure 1 (now Lines 251-5), and in a footnote in Table 1 and 4 of the Results section. Figure 1 has been modified to include study exits according to maternal HIV status. The proportion of missing data did not appear to be differential according to maternal HIV status.

27. In the CONCLUSION section, it opens by stating that “infant size is an important risk factor for early infancy morbidity and mortality in Africa”. I do not believe you will find a citation to report “size” as being associated with morbidity and mortality, rather lower birth weight has repeatedly been shown to be associated with increased morbidity and mortality. If you can find a “size” reference, please add it. Otherwise, please change to birth weight and provide citations. The term has been changed to low birth weight vs size as indicated, and a reference provided for the LBW and morbidity/mortality association; Lines 387-8.

28. In the DISCUSSION section, please clarify the clinical significance of the decreased birth weight and growth observed in this study. If these infants survived through six months of life, is this a clinically significant difference?

The differences in infant anthropometric measurements between groups (HIV-positive mothers vs. HIV-negative and mothers with low vs. high MUAC) were large from a clinical standpoint. For example, HIV-positive women gave birth to infants that weighed >200 g less than infants born to HIV-negative women. In a larger study, this magnitude of weight difference would likely correspond to poorer clinical outcomes beyond 6-months as birth weight is a powerful predictor of infant survival in this setting, however, without follow-up beyond 6-months we cannot be certain.

29. On Table 1, CD4 cell count is listed under the OVERALL column with a report of n=114. Yet the table footnotes are clear that this data is only for HIV-infected women. All data should be reported under HIV-infected women, without data in the OVERALL column.
The Table has been modified to clarify this further.

Also, the 3rd footnote is presented in the data table before the second footnote. Numbering and the legend below the table should be presented in order.
Correction made to footnote order.

30. Tables 2 and 3 are very challenging to follow. First, it may help the readers to present this data in landscape format, so that all columns have equal width and the 95% CIs appear on the same line. This would also allow all negative effect estimates to appear beside their numerical value. For example, if the reader scans table 3, they might assume the HIV-infected women might give birth to infants weighing 280.38 grams more than HIV-uninfected women.
Modified as suggested.

Secondly, in the section of MUAC tertiles, please add the ranges for each tertile for the reader’s benefit.
Corresponding tertile cut-off points have been added within the Table column.

Finally, with respect to the letter designated footnote of significant differences in the between group comparisons, I am unable to discern how to interpret “a” versus “ab” versus “b”. Can this be clarified?
A footnote has been added to Tables 2 and 3 indicting that values with different letters are significantly different (P<0.05), and a comma has been added to the “a,b” designation to indicate that this group is not significantly different from “a” or “b”.

31. On table 4, the prevalence of “WASTING” for HIV-exposed infants actually appears to be the prevalence of underweight (weight-for-age z-score of greater than 2 standard deviations below the norm of the reference population). If you look across the time periods, WAZ is not declining at the same rate as LAZ. Since wasting is a function of weight-for-length, if weight doesn’t decline at the same rate as length, the infant’s weight is becoming increasingly more proportional to length over time, indicating that while incidence of stunting may be increasing over time, wasting would be decreasing. Please check this for the entire table.
Corrected as indicated.

32. For Table 4, please provide the ranges for “low gestational MUAC” and “high gestational MUAC”.
A footnote indicating ranges has been added to Table 4.

33. In Table 5, it would indicate that each additional week of exclusive breastfeeding was associated with a decline in both WAZ and LAZ of 0.02. Intuitively, this does not make sense. Is the variable coded correctly in the model?
Data have been re-verified as correctly coded and analyzed, and the direction of the association is confirmed as correct. While the finding was not statistically significant and therefore we interpret that there is no association in this dataset between exclusive breastfeeding and WAZ or LAZ, we speculate that the fact that HIV-positive mothers exclusively breastfed their infants for a significantly longer duration may be contributing to this counterintuitive direction in the observed association. Additional wording noting this speculation has been added; Lines 361-4.

Minor Recommendations:

1. In the CONCLUSION section of the ABSTRACT, reference is made to maternal mid-upper arm circumference being associated with “suboptimal” birth and infant anthropometry. The
findings actually demonstrate that lower maternal MUAC is associated with lower birth and infant anthropometry. This is a more factual statement of the findings. The conclusion could incorporate the fact that this may place infants born to women with lower MUAC at greater risk for morbidity and mortality.

Modified as suggested; Lines 50-1.

2. The CONCLUSIONS section of the abstract concludes with the statement “Gestational mid-upper arm circumference has the potential to identify at-risk women in need of additionally health invention that may prevent negative fetal and infant consequences. You may want to consider revising this to read “Routine assessment of maternal mid-upper arm circumference has the potential to identify at-risk women in need of additional health interventions designed to optimize pregnancy outcomes and infant growth.”

Modified as suggested; Lines 53-4.

3. The final sentence of the CONCLUSIONS section of the ABSTRACT calls for further research to establish gestational mid-upper arm circumference reference ranges. It may also be appropriate to define a package of interventions that successfully improve MUAC in pregnancy.

Modified as suggested; Line 54-6.

4. In the second paragraph of the BACKGROUND section at line 70, there is a sentence which currently reads “While these indicators identify severe problems, they pregnant women with mild or moderate malnutrition may be unidentified”. Should the “they” be removed from this sentence?

Sentence has been modified; Lines 72-4.

5. In the final sentence of the second paragraph of the BACKGROUND section at line 75, it reads “…pregnancies that represent the crucial first step towards achieving better maternal, fetal and infant care….”. Should the “that” be removed from this sentence?

Modified as suggested; Lines 76-9.

6. In the STUDY POPULATION portion of the METHODS section at line 104 it is indicated that women were followed from “mid-gestation” through delivery. Given the comment above about the nebulous nature of “mid-gestation and that fact that some women were only followed from 34 weeks through gestational, might it be better to indicate that women were followed from time of enrollment through delivery?

The term mid-gestation has been removed throughout the revised manuscript.

7. There appears to be a typo at line 192 on page 10 in the MATERNAL CHARACTERISTICS portion of the RESULTS section where is states “or 3 triple therapy”. Should this simply be “triple therapy”?

This was correct, although to avoid confusion we have changed the listing order so that triple therapy follows number 2 in the list, and AZT monotherapy follows number 3; Lines 237-9.

8. The word “women” is likely missing from line 194 on page 10 in the MATERNAL CHARACTERISTICS portion of the RESULTS section, which current reads “Among who began ART during pregnancy….”.

Modified as suggested; Line 242.

9. In the INFANT GROWTH portion of the RESULTS section, reference is made to a rural cohort (line 220). This is actually a rural and semi-rural cohort.

Modified as suggested; Line 248.
10. In the INFANT GROWTH portion of the RESULTS section, there is likely a typo at lines 229 where it reads “…tertile associated with significantly lower WAZ at throughout infancy…”. The “at” should likely be deleted.  
Modified as suggested; Line 279.

11. In the DISCUSSION section at line 283, these women probably need multiple interventions, so intervention should be changed to interventions and “is” following the word consequences should be changed to “are” to achieve appropriate grammar.  
Modified as suggested; Line 339.

12. It is redundant to say in the DISCUSSION section starting at line 286 “implementing the most appropriate maternal intervention for these women”, and might be better worded as “implementing the most appropriate interventions for these women”.  
Modified as suggested; Lines 341-3.

Reviewer's report (#3)

Reviewer: Mathurin Tejiokem

The major comments

Methods:
Study population.
1) It will be important to know more about the setting. This will help clarify the population where the authors selected the 114 women during the eight months of this study. This will throw more light to the moment mothers were included in this study. This is an important point given the diversity of rural populations, and the comparatively limited data available. We have expanded the description of the study setting in the methods (Lines 98-108).

2) Among the eligibility criteria, there was “singleton birth”. Normally, it should be singleton pregnancy. If not, were there any morphological analysis apart from clinical exams to confirm singleton pregnancy? Was there a pre-inclusion phase? Did the authors pre-include during pregnancy and confirm after birth?  
Our approach was to include all pregnant women in the study, and then withdraw from all analyses if a multiple delivery was confirmed. This has been added to the methods to clarify in Lines 115-7.

3) The total sample size in each of the two groups involved in this analysis was not justified. Full justification of sample sizes with multiple statistical analyses techniques and two study populations (i.e. sometimes only maternal data included, other times infants only, other times both) in the manuscript text is difficult. The following calculations are relevant to this study:

Key outcome variable estimation of two-sample (unequal sizes) comparison of means sample size calculations based on proposed clinically meaningful differences; calculations apply to any two-group comparisons, i.e. HIV-exposed vs HIV-unexposed groups; HIV-positive vs HIV-negative.

Birth weight: assuming birth weight difference between groups=300g, standard deviation in either group=425g, ratio between groups=2, power=0.80, 2-sided significance=0.05 requires group 1=24 and group 2=48 subjects.
Birth length: assuming birth length difference between groups=1.25 cm, standard deviation in either group=2 cm, ratio between groups=2, power=0.80, 2-sided significance=0.05 requires group 1=31 and group 2=62 subjects.

Gestational age at birth: assuming gestational age at birth difference between groups=1.5 weeks, standard deviation in either group=2.5 weeks, ratio between groups=2, power=0.80, 2-sided significance=0.05 requires group 1=33 and group 2=66 subjects.

Birth MUAC: assuming birth MUAC difference between groups=0.75 cm, standard deviation in either group=1.2 cm, ratio between groups=2, power=0.80, 2-sided significance=0.05 requires group 1=31 and group 2=62 subjects.

Head circumference: assuming head circumference difference between groups=1.0 cm, standard deviation in either group=1.5 cm, ratio between groups=2, power=0.80, 2-sided significance=0.05 requires group 1=27 and group 2=54 subjects.

For one-way ANOVA (3-groups, i.e. gestational MUAC low, medium, high), with interest in medium to large effect sizes (by convention set at 0.25<f<0.40) assuming power=80%, statistical significance=0.05, a total sample size of 75 (approx. birth data) would be able to detect effect sizes f=0.37; a sample size of 111 (approx. maternal data) would be able to detect effect sizes of f=0.30.

The study was not powered to detect differences in proportions for pregnancy outcomes (stillbirth, LBW). Given the limited published data available from similar settings, we elected to report these data, but in the revised version we have removed the statistical p-value, and report only the absolute differences in the text and table. Example difference in proportions (unequal group sizes) that this study would be able to detect include: proportion 1=0.10, proportion 2=0.30 – difference of 20, assuming power=80%, statistical significance=0.05, group ratio=2, would require 35 and 69 subjects; for higher frequencies proportion 1=0.05 and proportion2=0.20 – difference of 15 would require 42 and 83 subjects.

For simple linear regression (one predictor), with interest in small to medium effect sizes (by convention set at 0.02<f<0.15), assuming power=80%, statistical significance=0.05, a total sample size of 74 would be able to detect an effect size of 0.11; a sample size of 101 would be able to detect an effect size of f=0.08.

For multiple linear regression (one predictor, 6 covariates), with interest in medium to large effect sizes (by convention set at 0.15<f<0.35), assuming power=80%, statistical significance=0.05, a total sample size of 73 would be able to detect an effect size of 0.22; a sample size of 97 would be able to detect an effect size of f=0.16.

Sample size calculations were not made for linear mixed effects regression.

4) Line 103: “For every HIV-positive woman enrolled, the recruitment goal included two HIV-negative women.” Were there any procedures to match these women? If yes, on which criteria? Please could you give precision on how HIV-negative women were selected? HIV-positive and HIV-negative women were not matched. HIV-positive and HIV-negative women were enrolled at approximately the same rate to avoid an imbalance in enrollment periods – i.e. season of enrollment. HIV-positive women attending the clinic for antenatal care were invited to enroll if they provided consent and were eligible according to the study eligibility criteria. We anticipated that HIV-negative retention would be more challenging, especially for the additional study-specific month 6 follow-up appointments and so we recruited more HIV-negative women. We have added to Lines 117-20 that study subjects were not matched.
Data collection:
5) Lines 146-149: breastfeeding variable: difficult to understand the relevance of this because difference between partial and predominant breastfeeding is not clarify and the authors did not define these terms.
We have added the definitions in the methods (Lines 183-6), and in a footnote in Table 5.

Discussion
6) One third of pregnancies endup as premature deliveries. This seems to be too much. What could explain this? This has not been discussed. Could this have an impact on the result?
There were a high proportion of preterm deliveries in this study; however, this is a figure similar to other Tanzanian estimates of preterm delivery.


We suspect this figure is affected by inaccuracies of gestational age estimations in the absence of ultrasound technology. While ultrasound would be preferable, the study resources were unable to provide the technology and training to include this. In order for the reader to be able to evaluate the data with this limitation in mind, we have attempted to provide a detailed description of the methods we used to estimate gestational age (Lines 156-60). Additionally, we have added a sentence (Lines 372-4) in the limitations section noting this potential misclassification.

The same observation with skin folds parameters which were finally not discuss.

We did not focus on skinfold thickness because our evidence suggested that triceps skinfold thickness had no advantages in terms of identifying at-risk mothers compared to MUAC, and since triceps skinfold thickness required more training than MUAC as well as a more expensive instrumentation requiring skinfold calipers. Overall, we thought it more important to focus on MUAC requiring only a tape measure and minimal training as being feasible to implement as part of antenatal care in a resource-restricted clinical setting.

General comment
I agree with the authors’ conclusion but I have one question in mind concerning the use of gestational MUAC as point of care test. Considering the fact that appropriate reference values are not yet established and that some authors reported that anthropometric measures for infants born to HIV-infected mothers are significantly lower than those for infants born to HIV-negative mothers because of direct HIV effect or concurrent illnesses or triple therapy effect, should we not directly consider HIV positive mothers as a risk group?
We agree that until the long-standing enigma of why HIV-exposed infants experience poorer growth outcomes compared to their never-exposed counterparts remains unresolved, and that maternal HIV seropositivity alone should be considered a risk factor for poor fetal or infant growth. However, it is also likely that all HIV-exposed infants are not at equal risk of poorer growth outcomes and with limited resources, perhaps they need and will most benefit from further attention. Our evidence suggests that using maternal MUAC in this and similar rural settings (where height boards and adult weigh scales are frequently absent, and where visually
identifiable changes in maternal weight may mask more subtle maternal malnutrition), may help identify pregnancies at greater risk, irrespective of HIV status.

We have modified the last sentence of the conclusion to include the important acknowledgement that maternal HIV-seropositivity alone is a risk factor: “Maternal nutritional status did not differ significantly according to HIV-status based on standard clinical and anthropometric indicators, however, HIV-exposed pregnancies and infants still fared worse in this rural setting and maternal HIV-seropositivity alone remains a direct risk factor for poorer fetal and infant growth”.

**Minor comments**

**Abstract:**
1) The study period and setting are not indicated. The process of selection of mother-infant pair is not mentioned. The study setting (rural/semi-rural Tanzania) and period (March to November, 2012) have been added to the Abstract, and the selection process of being invited to participate at antenatal clinic visits was included.

2) Line 30: It is noted that “Data was obtained by questionnaire”. Data here is plural so should read “Data were obtained”? Corrected.

**Background:**
3) Line 70: “While these indicators identify severe problems, they pregnant women”. This sentence is not clear, something is missing here. Please correct. This sentence has been modified in lines 72-4 to read: “While these indicators can identify severe maternal malnutrition and clinical complications, pregnant women with mild or moderate malnutrition may remain unidentified.”

**Methods:**
4) Line 106: Could you please present the infant vaccination schedule in Tanzania? Which infant vaccine is planned at 6 months of age? Tanzanian vaccinations were scheduled for months 1, 2, and 3. The 6-month visit was a study-specific appointment and did not correspond to a scheduled vaccination. We have clarified this in Lines 135-7: “Mother-infant follow-up visits corresponded to the Tanzanian infant vaccination visit, with the exception of a study-specific visit at 6-months. If a mother-infant pair failed to return for a follow-up visit, a field worker traveled to their last known address to invite them to return to the clinic for a rescheduled appointment.

5) Lines 104-105: was AZT+3TC+EFV the first line regimen in Tanzania? Were there any restrictions of this regimen in first three months of pregnancy? All women in this cohort were in their second or third trimester, and for these women AZT+3TC+EFV was a preferred first-line combination antiretroviral regimen in Tanzania at the
time of data collection. This sentence has been modified in Lines 145-7 to indicate: “Women with absolute CD4 cell counts <350 cells/µL were eligible for a first-line combination antiretroviral regimen, consisting of AZT + lamivudine (3TC) + efavirenz (EFV)."

7) Line 121: How many infants were tested? Were there any miscarriages, any deaths before testing or any lost to follow up? We have modified this section in Lines 148-52 to indicate: “HIV-exposed infants were prescribed daily nevirapine for six weeks, followed by HIV testing at the regional hospital laboratory at Bugando Medical Centre at 3-months of age using dried blood spot DNA-PCR. Out of the 38 HIV-exposed infants enrolled in the cohort at delivery, 32 (84%) tested negative for HIV at 3-months and 6 (16%) exited the study prior to HIV testing.”

Data collection:
8) Lines 123-124: “Self reporting” and then “questionnaires administered by the…” a bit ambiguous to me. Was it a guided self reporting? This has been clarified in Lines 155-6: “Demographic, medical history, and infant feeding data were obtained through questionnaires administered by a Swahili-speaking research nurse.”

9) The authors indicated how measures were done but nothing is stated concerning the person who conducted them. Was this carried out by the research nurse? We have added the following sentence in Line 160-1: “All maternal and infant anthropometric measurements were collected by trained research nurses.”

10) When were the data collected in pregnant women? This sentence has been modified in Line 161-3 to state: “Gestational anthropometric measurements were collected upon enrollment that corresponded to the second or third trimester of pregnancy.”

Results
11) Lines 179-180: This should be checked. Does not correspond with what is on the tables. The two numbers for gestational age at enrollment were incorrectly switched in Table 1 for the HIV-positive and HIV-negative groups. With the Table 1 correction that corresponds to the gestational ages that have been double-checked against the raw data, the text data interpretation now correctly corresponds to the data in the revised Table 1.

12) Line 189: “...may have been due infrequent severe ...” something is missing This sentence has been modified in Line 235-7 to read: “Among HIV-positive women, advanced immunosuppression was rare as 3/4 had CD4 ≥350 cells/µL and almost half (46.5%) had normal absolute CD4 cell counts (≥500 cells/µL)”

13) Line 194: “Among who began ART...”: not clear, please complete This sentence has been modified in Line 242 to indicate, “Among those women who were prescribed AZT...”

14) Table 2: correct please the unit of birth weight: not cm. the same observation in Table 3 We have corrected “cm” to “g” as the unit corresponding to birth weight in Tables 2 and 3.

15) Figure 1: considering the details presented before the 3 months follow up synthesis, the number of infants analysed should be 92/100 giving 92%. One maternal-infant pair had been incorrectly labelled as lost to follow-up after 3 months; however, they were in fact lost to follow-up prior to the 3 month study visit. This has been
corrected in the revised Figure 1.

Acknowledgements

16) Line 341: correct the term “cohort”
The spelling of this word has been corrected.